

PN IM2280**50 tests
20 µL/test****CD45 - FITC
GLYCO A - PE****IO Test®**
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY**CD45**

The CD45 family of transmembrane glycoproteins is comprised of five different isoforms with molecular masses ranging from 180 to 220 kDa.

The different isoforms are generated by alternative splicing of three exons encoding peptide segments designated A, B and C.

All the isoforms have the same eight amino-terminal amino acids, which are followed by the various combinations of A, B and C peptides. The remaining portion of all the CD45 isoforms are the same (1).

CD45 is expressed on the surface of all human leukocytes and plays an important regulatory role in T-cell activation.

The J33 monoclonal antibody binds to all the CD45 isoforms on human leukocytes.

This J33 antibody has been assigned to the CD45 cluster of differentiation at the 3rd International Workshop on Human Leukocyte Differentiation Antigens in Boston (1986).

Glycophorin A

Glycophorin A is a sialoglycoprotein, expressed on human red blood cell membranes and erythroid precursors, including proerythroblasts, and reticulocytes.

REAGENT

CD45	GLYCOPHORINE A
J33	11E4B7.6
IgG1 mouse	IgG1 mouse
X63-Ag8.653 x Balb/c spleen cells	NS1 x Balb/c spleen cells

Source Ascites Fluid**Purification** Ion exchange or affinity chromatography**Conjugations** FITC: Fluorescein isothiocyanate (FITC) is conjugated at 5 - 8 moles of FITC per mole of IgG.

Excitation wavelength: 488 nm

Maximum emission wavelength: 525 nm

Main emission color: Green

PE: R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG.

Excitation wavelength: 488 nm

Maximum emission wavelength: 575 nm

Main emission color: Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.**APPLICATION**

Flow Cytometry.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.

2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.

3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes

4. Do not use antibody beyond the expiration date on the label.

5. Do not expose reagents to strong light during storage or incubation.

6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 °C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25 °C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.

Assay volume: 20 µL/5 x 10⁵ cells / test or 100µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of an appropriate mixture including leucocyte and red blood cell suspensions stained with CD45-FITC/Glyco A-PE dual color reagent (PN IM2280). This mixture allows to mimic the presence of red blood cells in a lyzed normal whole blood cell immunostaining. Leucocytes labeling is obtained separately on a lyzed normal whole blood sample. Red blood cells labeling is obtained separately on the same (but not lyzed) normal whole blood sample. Acquisition is done on the appropriate "leucocytes/erythrocytes" cell suspension mixture. Gate is on a large region around lymphocytes (on Side Scatter versus Forward Scatter diagram) including than red blood cells.

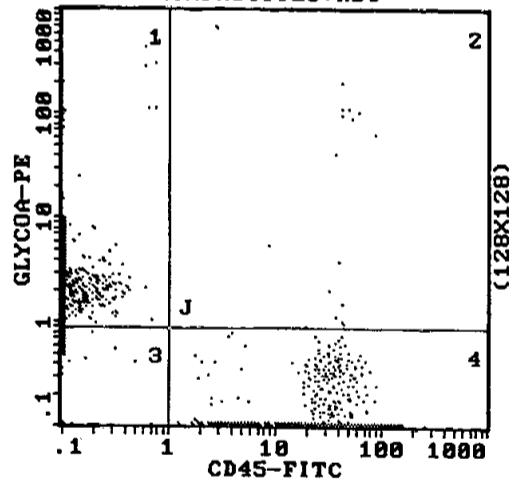
*Upper left quadrant (1) contains CD45- Glyco A+ events representing the red blood cells fraction of the gated events.

*Upper right quadrant (2) defines double positive CD45+ Glyco A+ events that should normally not happen.

*Lower left quadrant (3) contains double negative events CD45- Glyco A-.

*Lower right quadrant (4) contains CD45+ Glyco A - events representing the lymphocyte fraction of the gated events.

Acquisition is with a COULTER R EPICS R XL flow cytometer. Analysis is with the XL SYSTEM II TM software.

LYMPHOCYTES+RBC

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**COULTER**

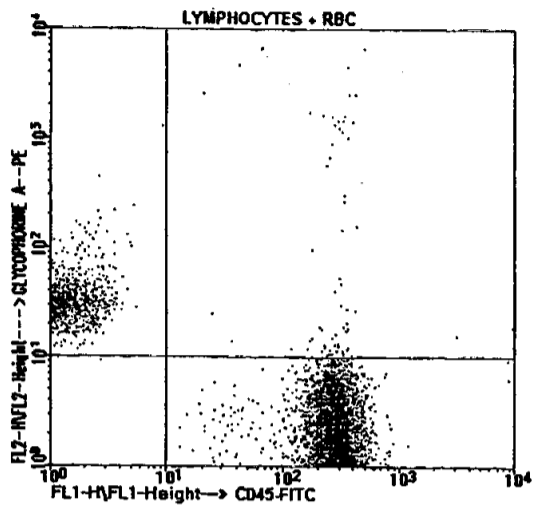
PARTNERS IN CELL ANALYSIS

**IMMUNOTECH**
A COULTER COMPANY

PN IM2280 CD45 - FITC
50 tests
20 µL/test
GLYCO A - PE

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Acquisition is with a Becton Dickinson FACScan™ flow cytometer.
 Analysis is with the LYSYS II™ software.



SELECTED RESEARCH REFERENCES

- 1-[285] Serra-Pages, C., Morimoto, C., Schlossman, S.F., Salto, H., Streuli, M., "Characterization of CD45 mAb", 1995, *Leucocyte Typing V, White Cell Differentiation Antigens*, Schlossman, S.F., et al., Eds., Oxford University Press, 389-391.
- 2-[151] Chasis, J.A., Reid, M.E., Ronald, H.J., Mohandas, N., "Signal transduction by glycoprotein A: role of extracellular and cytoplasmic domains in a modulatable process", 1988, *J. Cell Biol.*, 107, 1351-1357.
- 3-[152] Catimel, B., Wilson, K.M., Kemp, B.E., "Kinetics of the autologous red cell agglutination test", 1993, *J. Immunol. Meth.*, 165, 183-192.
- 4-[286] Cobbold, S., Hale, G., Waldmann, H., "Non-lineage, LFA-1 family, and leukocyte common antigens: new and previously defined clusters", 1987, *Leucocyte Typing III, White Cell Differentiation Antigens*, A.J. McMichael, 788-803.

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Manufactured by



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130, avenue de Lattre de Tassigny B.P. 177 13276 MARSEILLE Cedex 9 (FRANCE)
 Tel : (33) 4 91 17 27 00 - Fax : (33) 4 91 41 43 58 - e-mail : abmarket@immunotech.fr